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GROWTH PERFORMANCE, HAEMATOLOGY AND BIOCHEMICAL CHARACTERISTICS OF *Clarias gariepinus* JUVENILE FED CHICKEN EGG SHELLS AS REPLACEMENT FOR DICALCIUM PHOSPHATE

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ABSTRACT

The effects of substituting di-calcium phosphate (DCP.) with chicken egg shell (CES.) on the growth, haematology and biochemistry of *Clarias gariepinus* were investigated for ten weeks in a feeding experiment. One hundred and fifty juveniles of *Clarias gariepinus* were fed at varying inclusion levels of 0%, 25%, 50%, 75% and 100% CES meals. Significant difference occurred ($P < 0.05$) in the mean weight gain (MWG) (35.54±5.69), average feed intake (AFI) (0.8029±0.0967) and protein efficiency ratio (PER) (1.48±0.18) of the fish, with the highest values in the fish fed 25%CES. while the lowest occurred at 75% substitution level.. In the haematological parameters, red blood cell (RBC), packed cell volume (PCV) and haemoglobin (Hb) levels were highest in the 50% CES. WBC values recorded in the fish fed with the test ingredients were significantly higher ($P<0.05$) than that of the control. The biochemical analysis showed that the urea values observed in the fish fed with the test feed were significantly higher ($P<0.05$) than that of the control with the highest value recorded in diet 50% CES. In conclusion, chicken egg shell could be substituted with di-calcium phosphate (DCP) up to 25% and or 50%level of substitution in *Clarias gariepinus* diet without any negative effects on the growth and feed efficiency.

Keywords: Chicken egg shell, di-calcium phosphate, substitution, *Clarias gariepinus*, diet

INTRODUCTION

Aquaculture, which involves the farming of fish for nutritional and economic benefits as well as food security and income generation [illegible] *al.,* 2012). The need arose from the decrease in supply from ocean fisheries as a result of over-fishing, habitat destruction and pollution (Adedeji and Okocha, 2011). The level of intensification witnessed in recent times has raised several issues that need to be addressed for the sustainability of the industry (Adelakun *et al.,* 2012).

Good nutrition in animal production systems is essential to economically produce a [illegible] In fish farming, nutrition is critical because feed represents 40-50% of the production costs (Steven and Helfrick, 2009). Jamu and Ayinla (2003) reported that feed accounts for at least 60% of the total cost of fish production in Africa, which to a large extent determines

the viability and profitability of fish farming enterprise. As aquaculture becomes intensive, most farmers in Africa depend largely on imported fish feed from European countries for the productivity and sustainability of the industry. For example, in Nigeria an estimated 4,000 tons of quality fish feeds are imported into the country each year (AIFP, 2004). This has contributed in no small way in increasing the total cost of production which will ultimately translate to high cost of fish, thereby making it expensive for the teeming population of the poor people living in Sub-Saharan Africa. This has motivated the research for local, cheap and unsuitable for direct human consumption products as alternative energy feed for *Clarias gariepinus* that aim to reduce the cost of production without compromising fish quality.

Calcium and phosphorus are two of the major constituents of the inorganic portion of feeds. Quantitatively, calcium and phosphorus function primarily as structural components of hard tissues (e.g. bone, exoskeleton, scales, and teeth), muscle function, proper nerve impulse transmission, osmoregulagulation, and as a cofactor for enzymatic processes (National Research Council, 1993). Dicalcium phosphate is a feed additive incorporated into feed to provide calcium and phosphorus which are important minerals for growth. It is derived from bones of animals which undergo several chemical processes (in which a large amount of protein is lost) before it can be safe for usage. A breach in these chemical processes will complicate the end result, so the various steps must be strictly followed (Waldroup, 1997). Eggshells contain calcium and trace amounts of other micro elements, i.e. magnesium, boron, copper, iron, manganese, molybdenum, sulphur, silicon and zinc. Eggshell calcium is probably the best natural source of calcium and it is about 90% absorbable (Bee, 2011).Examining the series of processes to acquire dicalcium phosphate and the simple process of milling the eggshells of chicken, one would note that the labour involved is minimal, nutrients are being preserved and invariably, cost is reduced in favour of the egg shells. The aim of this study is to investigate the growth performance, haematological and biochemical parameters of *Clarias gariepinus* fed with milled chicken egg shells incorporated at varying levels in catfish juvenile diets for 12 weeks to determine the appropriate replacement level (s) of dicalcium phosphate.

MATERIALS AND METHODS

Collection of specimen: One hundred and fifty juveniles of catfish *Clarias gariepinus* were bought from Treasures Bath Farm in Ikotun, Lagos State and transported to the Marine Research Laboratory of the Department of Marine Sciences, University of Lagos, Akoka, Lagos.

Experimental procedure: The fish were allowed to acclimatize in holding tanks for 14 days during which they were fed on commercial diet (Coppens: 2mm). At the end of the acclimatization period, the fish were starved for 24 hours prior to the commencement of the experiment to enable them empty their guts. Then 10 juveniles of *Clarias gariepinus* were transferred into each of the experimental plastic tanks. They were sorted into uniform size range and were allotted randomly into 15 plastic aquaria tanks. The tanks were labelled according to the percentage of egg shell inclusion level as indicated below:

Tank 0: 100% DCP and 0 % egg shell (Control tank)

Tank 1: 75% DCP and 25% chicken egg shell
Tank 2: 50% DCP and 50% chicken egg shell
Tank 3: 25% DCP and 75% chicken egg shell
Tank 4: 0% DCP and 25% chicken egg shell

The tanks were labelled and set in triplicates per diet treatment. Suitable water conditions were maintained by cleaning the tanks and constant changing of water which took place at regular intervals (every two days for the first four weeks then everyday afterwards). The fish were fed twice each day (morning-9:00am and evening 6:30 pm) for 10 weeks. Feeding response was monitored.

Table 1 showed the different feed ingredients used in formulating the various diets. The feeds were formulated using Pearson Square method (38% as the crude protein).

Table 1: Feed composition of the experimental diets for the juvenile *Clarias gariepinus*

Items	Percentage composition				
	0%CES Treatment 1(Control)	25% CES Treatment 2	50% CES Treatment 3	75% CES Treatment 4	100% CES Treatment 5
Indomie	15.77	15.77	15.77	15.77	15.77
Maize	15.77	15.77	15.77	15.77	15.77
Groundnut cake meal	21.62	21.62	21.62	21.62	21.62
Soya bean meal	21.62	21.62	21.62	21.62	21.62
Fish meal	21.62	21.62	21.62	21.62	21.62
Vitamin C	0.1	0.1	0.1	0.1	0.1
Fish premix	0.5	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3	0.3
Oil	0.7	0.7	0.7	0.7	0.7
Di- calcium phosphate (DCP)	2.0	1.5	1.0	0.5	0
Chicken Egg shell (CES)	0	0.5	1.0	1.5	2.0
% Total	100	100	100	100	100

CES = Chicken Egg Shell

Growth and nutrient utilization parameters: The mean weight of fish in each tank was determined at the beginning of the experiment and at the end of every week (for eight weeks). The weight of all the fish in each tank was measured using the weighing scale (OHAUS MODEL CS 5000, CAPACITY 5000X2G) and mean values were calculated. The following indices were used to determine the biological evaluation of growth performance and nutrient utilization of the fish.

utilization of the fish.

i) Mean Weight Gain (MWG) = $\frac{W_f - W_i}{\text{Number of days}}$

Where W_f = final average weight (g)

W_i = initial average weight (g)

ii) Specific Growth Rate (SGR): = $\frac{\text{Loge}_{W_f} - \text{Log e}_{W_i}}{\text{Number of days}} \times 100$

Where W_f = final average body weight

W_i = initial average body weight

iii) Food Conversion Ratio (FCR): = $\frac{\text{Feed intake (g)}}{\text{Total weight gain (g)}}$

iv) Protein Efficiency Ratio (PER): = $\frac{\text{Mean weight gain (g)}}{\text{Protein intake}}$

v) Relative Growth Rate (RGR) = $\frac{\text{Mean weight gain (g)} \times 100}{\text{Initial average body weight (g)}}$

vi) Protein Intake : = Feed fed x crude protein of the feed.

Blood collection and blood analysis: Five fish were taken (from each tank) out individually in a small hand net and placed belly upward. Blood sample was collected from the heart of the fish using 2ml syringe. 1ml of blood sample was collected from the fish (five) in each tank and dispensed into heparinized tube and was preserved in ice pending the time for analysis in order to prevent the blood from coagulating.

The haematological and biochemical parameters analyses carried out included: Total erythrocytes count (RBC), haematocrit (Hct or PCV), haemoglobin concentration (Hb), haematological indices (MCHC, MCH and MCV), urea, creatinine, albumin, total protein, alkaline phosphatase, triglyceride and globulin. The haematological parameters were analysed at the medical laboratory in Lagos University Teaching Hospital (LUTH) using Mind Ray BC-2800 haematology analyser (China) while the biochemical analyses were done using laboratory procedures.

Statistical analysis: Data collected were subjected to analysis of variance (ANOVA). Comparison among diet means was carried out using Duncan Multiple Range Test at a significant level of 0.05.

RESULTS

(i) Growth and nutrient utilization:

The results obtained from the growth response and nutritional utilization of the fish fed with chicken egg shell diets are shown in Table 2. The highest MWG was observed in the fish fed 25% substitution level of the chicken egg shell while the least were in (75%). However, there was no significant difference in the PI, SGR, RGR and the FCR values of the fish from all diet types (P>0.05).

(ii) Haematological analysis

Table 3 shows the haematological changes in test fish and the control fish. The mean of the haematological parameters ranged as follows: Haemoglobin (Hb) - 4.80 ± 0.42 to 10.00 ± 0.57 g dL^{-1}, Packed Cell Volume (PCV) - 18.00 ± 0.00 to 32.50 ± 2.12 %, Red blood cell - 1.3±0.28 to 3.1±0.42, White Blood Cell (WBC) - 11600 ± 15.56 to 17000 ± 2404.16 ccm, Mean Corpuscular Haemoglobin (MCH) - 3.50 ± 0.28 to 4.40 ± 0.28 pg, Mean Corpuscular Haemoglobin Concentration (MCHC) - 25.26 ± 0.82 to 38.88 ± 2.84 (g/l). Mean Corpuscular Volume (MCV) - 90.00 ± 3.92 to 146.15 ± 2.59 fl.

(iii) Biochemical analysis

The mean values of the biochemical parameters of the fish fed with the various diets are presented in Table 4. The mean urea ranged from 18.32 ± 1.85 to 35.62 ± 4.32 $mgdL^{-1}$ while mean creatinine value had 0.20 ± 0.00 mg dL^{-1} as its lowest value and 0.70±0.14 mg dL^{-1} as the highest value. Mean total protein ranged from 3.80 ± 0.14 to 4.50 ± 0.14 $mgdL^{-1}$. Albumin had its lowest value as 1.90 ± 0.00 and 2.00 ± 0.42 mg dL^{-1} as its highest value. Triglyceride values ranged from 90.47 ± 3.08 to 123.46 ± 2.97 mg dL^{-1} and alkaline phosphatase from 4.00 ± 0.00 to 8.00 ± 1.41 mg dL^{-1}.

Table 2: Growth and nutrient parameters of the control *Clarias gariepinus* and those fed with chicken egg shell

Parameters	Trt1	Trt2	Trt3	Trt4	Trt5
Final weight(g)	52.11±3.68ab	56.51±6.22b	47.18±8.0ab	39.82±3.55a	48.10±9.23ab
Initial weight(g)	18.07±3.65a	20.97±4.24a	17.40±3.40 a	16.10±1.82 a	20.47±4.84 a
MWG (g/day)	4.86±0.38 b	5.08±0.81 a	4.25±0.71 ab	3.39±2.61 a	3.95±0.65 ab
TFI (g/day)	7.54±0.44 b	8.02±0.97 b	6.66±1.13 ab	5.84±0.20 a	6.88±1.15 ab
AFI (g/fish)	0.7539±0.441 b	0.8029±0.0967 b	0.6659±0.1131 ab	0.5843±0.0201 a	0.6880±0.1154 ab
SGR (%/day)	1.530±0.2154 a	1.4292±0.2617 a	1.4289±0.9607 a	1.2962±0.1163 a	1.2314±0.0907 a
RGR (%/fish)	27.71±6.05 a	25.01±7.50 a	24.62±2.60 a	21.19±2.83 a	19.58±2.14 a
FCR	1.5518±0.0411 a	1.5930±0.1680 a	1.5725±0.1717 a	1.7352±0.1550 a	1.7450±0.1217 a
PI	24.50±0.1513 a	24.04±2.64 a	24.37±2.77 a	22.02±1.95 a	21.85±1.50 a
PER	1.39±0.08 b	1.48±0.18 b	1.23±0.21 ab	1.08±0.04 a	1.27±0.21 ab

Means with the same superscripts along the row are not significantly different (p>0.05)

PI = Protein intake, PER = Protein efficiency ratio. MWG = Mean weight gain, TFI = Total feed intake, AFI = Average feed intake, SGR = Specific growth rate, RGR = Relative growth rate, FCR = Feed conversion ratio.

Table 3: Haematological parameters of the control *Clarias gariepinus* and those fed with chicken egg shell.

Parameters	Trt1	Trt2	Trt3	Trt4	Trt5
PVC %	22.00±2.83 a	18.00±0.00 a	32.50±2.12 b	19.00±1.4 a	21.00±2.83 a
RBC ɤ 109/L	1.8±0.28 a	2.0±0.57 a	3.1±0.42 b	1.3±0.09 a	1.6±1.4 a
Hb (g/dL)	6.80±0.42 b	7.00±0.28 b	10.00±0.57 c	4.80±0.42 a	7.00±0.42 b
MCV(fl)	122.10±2.97 c	90.00±3.92 a	106.50±1.95 b	146.15±2.59 d	131.25±5.63 c
MCHC(g/l)	30.91±1.12 ab	38.88±2.84 c	30.30±.1.90 ab	25.26±0.82 a	33.33±3.30 bc
MCH(Pg)	3.60±0.14 a	3.50±0.28 a	3.70±0.28 a	3.70±0.00 a	4.40±0.28 b
WBC(ccm)	11600±15.56 a	14400±1838.48 ab	17000±1131.37 b	17000±2404.16 b	15500±848.53 ab

Means with the same superscripts along the row are not significantly different (p>0.05) Means with the same superscripts along the row are not significantly different (p>0.05)
PCV = Packed Cell Volume, RBC = Red Blood Cell count, Hb = Haemoglobin, MCV = Mean Cell Volume, White Blood Cell count = WBC, Hb = Haemoglobin Concentration , Mean Corpuscular Haemoglobin (MCH), and MCHC = Mean Corpuscular Haemoglobin Concentration. PCV = Packed Cell Volume, RBC = Red Blood Cell count, Hb = Haemoglobin, MCV = Mean Cell Volume, White Blood Cell count = WBC, Hb = Haemoglobin Concentration , Mean Corpuscular Haemoglobin (MCH), and MCHC = Mean Corpuscular Haemoglobin Concentration.

Table 4: Plasma biochemical parameters of the control *Clarias gariepinus* and those fed with chicken egg shell

Parameters	Trt1	Trt2	Trt3	Trt4	Trt5
Creatinine (mg dL-1)	0.40±0.14ab	0.40±0.14 ab	0.20±0.00 a	0.70±0.14 b	0.35±0.07 a
Urea (mg dL-1)	18.32±1.85 a	24.45±3.05 a	35.62±4.33 b	21.79±1.32 a	23.51±0.04 a
Albumin(mg dL-1)	1.70±0.28 a	1.70±0.14 a	2.00±0.42 a	1.90±0.00 a	1.90±0.14 a
Total protein(g dL-1)	4.10±0.42 ab	3.80±0.14 a	4.00±0.00 ab	4.20±0.14 ab	4.50±0.14 a
Alkaline phosphatase (I UL-1)	8.00±1.41 b	4.00±0.00 a	8.00±0.00 b	8.00±1.41 b	6.00±0.00 ab
Triglyceride(mgdL-1)	123.46±2.97 b	90.47±3.08 a	119.62±2.35 b	97.69±3.22 ab	106.54±5.63 b
Globulin(g L-1)	2.40±0.14 ab	2.0±0.14 a	2.0±0.42 a	2.30±0.14 ab	2.60±0.00 b

Means with the same superscripts along the row are not significantly different (p>0.05)

DISCUSSION

The feeding trial revealed that juvenile *Clarias gariepinus* mean weight (16.10±1.82 to 20.47±4.84) responded to all the diets. There was significant difference ($P < 0.05$) in the MWG with the highest value in the fish fed diet (25% C.E.S). This also reflected in the values of the AFI and PER with the highest values at this level of substitution. It could be inferred that more feed was consumed per fish fed with this diet. This result seems to indicate the level of feed palatability, with reduced feed intake at higher concentrations of the test ingredient. This observation is contrary to the result from the work done by Ayoola and Maduekwe (2012) where lowest values of MWG and PER were recorded in fish fed at 25% substitution level of *Mytilus edulis* shell meal diet.

Blood is a good indicator in determining the health of an organism (Joshi *et al,*. 2002). It has been documented that different factors are effective on the haematological and biochemical parameters of fish from which the species, environmental condition, age, maturation and nutrition are very important (Ross and Ross, 1999). Materials on haematological and blood biochemical reference range of juvenile *Clarias gariepinus* are limited. Except haemoglobin, all other haematological parameters measured in this study were out of range with respect to the recommended physiological ranges reported for *Clarias gariepinus broodstock (Akinrotimi and Gabriel, 2012). This could be as a result of the fact that they are broodstocks cultured in water recirculatory system, unlike in the juveniles in this study.*

The PCV, RBC, Hb and MCH values of fish fed diet (25% CES) are closely related and are not significantly different ($P>0.05$) from the control's. RBC production, PCV and Hb levels were highest in diets (25% & 50% CES), this could be as a result of increase in blood cell production from the bone marrow. The PCV, MCV and RBC values are within the ranges observed in the works of Ayoola and Maduekwe, (2012). The WBC values recorded in the fish fed with the test ingredients are significantly higher ($P<0.05$) than that of the control, this could be as a result of the immune-modulatory effect of the high Sialic acid content in the chicken eggshell.

It has been reported that serum biochemical constituents are positively correlated with the quality of the diet (Brown and Clime, 1972; Adeyemi *et al*., 2000). The albumin values show no significant difference ($p>0.05$). The creatinine, alkaline phosphatase and triglyceride values are in close range to the results of Ayoola and Maduekwe, (2012). Awosanya *et al.,* (1999) demonstrated the dependence of blood protein on the quantity and quality of dietary protein. The level of urea and creatinine is a measure of tissue degeneration, which is a sign of tissue weardown (Ranjna, 1999). In this research, the urea values observed in the fish fed with the test feed were significantly higher ($P<0.05$) than that of the control (with the highest value recorded in diet (50% ES). These values correlate with the values reported in Ayoola, (2011).

In conclusion, the results obtained from this study and observations discussed show that chicken egg shell could be substituted for dicalcium phosphate (DCP) up to 25% level of substitution in juvenile *Clarias gariepinus* diet without any negative effects on the growth of the fish and its feed utilization efficiency.

REFERENCES

Adedeji, O.B., Okocha, R.C. 2011.Constraint to Aquaculture Development in Nigeria and Way Forward. *Journal of Applied Sciences Research, Nigeria*.**7**(7): 1133-1140

Adelakun, K.M., Ogundiwin, D.I.,Ajagbe, S.O., Ijagbemi A.B. 2012. Sustainable Aquaculture Practise: A Profitable Venture in Nigeria. Federal College of Wildlife Management, New Bussa, pp 265-272.

Adeyemi, O. A., Fashina, O. E., Balogun, M. O. 2000. Utilization of full-fat Jatropha seed in Broiler diet: Effect on haematological parameters and blood chemistry. In: Proc 25th Annual Conference Nig. Soc. Anim. Prod. (NSAP) 19th -23rd March, 2000, Umudike, pp: 108-109.

AIFP 2004. Inventory of feed producers in Nigeria. Published by Aquaculture and Inland Fisheries Project. Annex II of the National Special Program for Food Security with the Agriculture Development Program in all states and FCT Abuja, Nigeria. pp.1-8.

Akinrotimi, O.A., Gabriel, U.U 2012. Haematological Profiles of *Clarias gariepinus* Brood Fish Raised in Water Recirculating Aquaculture System. Advances in Agriculture, Sciences and Engineering Research. 2 (2) : 97 – 103.

Awosanya, B., Joseph, J.K., Apata D.F., Ayoola M.A. 1999. Performance, blood chemistry and carcass quality attributes of rabbit. *Int. Trop. J. Anim. Sci*. **2:** 89-96.

Ayoola, S.O. 2011. Haematological Characteristics of *Clarias gariepinus* (Burchell, 1822) Juveniles Fed with Poultry Hatchery Waste. *Iranica Journal of Energy & Environment*.**2**(1): 18 -23.

Ayoola, S.O., Maduekwe, I.A. 2012. Biochemical and haematological responses of *Clarias gariepinus* (Burchell, 1822) Juveniles Fed with Diets containing *Mytilus edulis* shell at varying levels. *Journal of Fisheries and Aquatic Science*.**7**(6): 468-474.

Brown, J. A., Clime, T. R. 1972.Nutrition and haematological values. *Journal of Animal Science*.35: 211-218.

Burley, R.W., Vadehra, D. V. 1989. The egg shell and shell membranes: Properties and synthesis. The Avian Egg, Chemistry and Biology. John Wiley, New York.25–64 pp.

Jamu, D.M., Ayinla, O.A. 2003. Potential for the development of aquaculture in Africa *NAGA* **26**:9-13.

Joshi, P. K., Bose, M. and Harish, D. (2002). Haematological changes in the blood of *Clarias batrachus* exposed to mercuric chloride. *Ecotoxicological Environmental Monitoring* **12** (2): 119- 122

Nakano, T., Ikama, N.I. and Ozimek, L. (2003). Chemical composition of egg shell and shell membranes. *Poult. Sci*.**82**: 510-514.
National Research Council. *Nutrient Requirements of Fish*. Washington D.C; National Academy Press (1993).

Nwanna, L.C., Adebayo, I.A. and Omitoyin, B., (2009). Effect of graded levels of phosphorus on growth and mineral concentration in giant African catfish, *Heterobranchus bi-dorsalis*. *African Journal of Biotechnology*.**8**16): 3947-3953.

Ranjna, C. (1999). Practical Clinical Bio-

chemistry Method and Interpretation, Jaypee Brother Medical publishers Ltd. New Delhi, *alcium and Phosphorus sources for poultry feeds.* University of Arkansaas, Fayetteville, AR UIndia. 267pp.

Ross, L.G. and Ross, B. (1999). *Anasthetic and Sedative Techniques for Aquatic Animals.* 2nd Edn., Blackwell Science, Oxford, UK., ISBN: 0-63205252X, 176pp.

Steven, C. and Helfrick, L.A (2009). Understanding Fish Nutrition, Feeds, and Feeding. Department of Fisheries and Wildlife Sciences; Virginia Tech. Aquaculture, 420-456:1-4 Waldroup, P.W. (1997). *C*SA, 21pp.

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